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# THE OCCURRENCE OF RABBIT VIRAL HAEMORRHAGIC DISEASE (RVHD) IN EGYPT (With 3 Figures)

By

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تفشي مرض النزف الدموي الفيروسي في الأرانب في مصر

في شاء سنة ١٩٩٢ بمحافظة أسيوط لوحظ تفشي مرض معدي شديد المشراوة والحدة في الأرانب الكبيرة • فقد حدثت عدة أوبلة للمرض في تجيعات الأرانب الستأنية وكانت نسبة الوفيات ١٠٠٪ في أحد التجععات التي بها ٢٦ أونب بينما كانت نسبة الوفيات ١٠٠٪ في تجمع آخر يحتوي على ٢٩ أرنبة كبيرة وكان المرض شديد التأثير وتفاوت شكله من الطور قبل الحاد إلى الطور الحاد • وقد لوحظ في تلك الأوبلة من وتفاوت شكله من الطور قبل الحاد إلى الطور الحاد • وقد لوحظ في بعض الأرانب شوهمات أن الأرانب تموت فجأة بدون ظهور أعراض اكلينيكية وفي بعض الأرانب شوهمات أنزفة دموية رغوية عند فتحة الأنف والمهبل أحيانا • وأظهر التشريح المرضي للأرانسب المنت مخاط مدم في القمية المهواثية مع تغير في لون الكيد مع قلة في مقدرة الدم على التجلط في القلب ١٠هذا وقد تم عبل السفات الباثولوجية للمرض وكذلك تشخيص المسرض في العمل • وأجريت العدوى المناعية للمرض في الأرانب القابلة للاصابة ونتج عن ذلك موت الأرانب خلال ٢) - ١٦ صاعة بعد العدوى المناعية مع ظهور صفات تشريحية مرضية مثل التي شوهدت أثناء الوباء الطبيعي للمرض •

#### SUMMARY

A highly fatal infectious disease was obtained in adult rabbits in Assiut area during winter of 1992. Many outbreaks of the disease occurred in domestic rabbit colonies. The mortality rate was 100% among a rabbit colony of 26 rabbits and it was 96.5% in another colony of 29 adult rabbits. The course of the disease was drastic and the clinical symptoms ranged between peracute and acute picture. Infected rabbit died suddenly without any observed clinical manifestations. Haemorrhagic foamy discharge from the nostrils and vagina was observed in few cases. Post mortem findings included bloody mucous in the trachea, discoloured liver. The diagnosis of the disease was relied on the epidemiological observation and by detection of the virus particles in hepatic cells by electron microscopy. Experimental infection of adult rabbits was carried out by organ suspensions.

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Infected rabbit died within 42 to 66 hours, with the same <u>post mortem</u> lesions as those observed in natural outbreaks. The organ suspensions of the diseased and experimentally infected animals showed a haemagglutination titer using type 0 human and chicken erythrocytes. Convalescent rabbit serum completely abolished haemagglutination in haemagglutination-inhibition test, whereas normal rabbit serum had no inhibitory effect.

#### INTRODUCTION

Nowadys, rabbits has some advantages over other herbivorous and omnivores, together with their importance for meat production in developing countries. Recently one of the most fatal diseases affecting rabbits is viral haemorrhagic disease. In 1984, the disease was reported in many regions of China (LIU et al., 1984; PU et al., 1984 and SHENG et al., 1985). The first report of the disease in Europe was in Italy in 1986, it has been reported subsequently from France, Bulgaria, Poland, Czechoslovakia and Germany (BOUJON et al., 1989 and OHLINGER et al., 1989). According to GREGG and HOUSE (1989) the disease caused an outbreak of highly fatal course in rabbits in Mexico city and rapidly spreaded to 13 states. Concerning Egypt, references about this disease up till now appear to be lacking.

The disease is characterized by an acute onset with fever, rapid respiration, sudden deaths and a high mortality rate. The pathological changes include severe generalized circulatory dysfunction and marked degeneration of parenchymatous tissue (XU and CHEN, 1989 and KOLBL et al., 1990). CAO et al. (1986) suggested that the virus is a new member of the Picornaviridae. Furthermore, the electron microscopy revealed that the viral particles resemble calicivirus (GRANZOW et al., 1989 and CAPUCCI et al., 1990).

In Egypt sudden deaths among rabbit farms during winter season of 1992 at Assiut area suspected to be "viral haemorrhagic disease" were observed. Therefore, the present work was carried out to study this problem concerning the clinical and pathological manifestations of this newly recognized disease.

# MATERIAL and METHODS

Severe deaths with unusual symptoms in numerous rabbitary, at Assiut area during winter season of 1992 were observed. Post mortem examination was performed on all dead animals. Lungs and liver were taken as a material for bacterial and viral demonstration, and to prepare antigen for haemagglutination test as well as for reproducing the disease in susceptible animals by the bacteria free suspension of these organs.

Light and electron microscopy: Samples for electron microscopy were fixed in 5% glutaraldehyde, postfixed with 1% osmium tetraoxide, dehydrated with ascending grades of ethanol and embedded in epon 812. Semithin sections were obtained and

stained with 0.25% toulidine blue for light microscopy. Representative fields were selected for transmission electron microscopy. Ultrathin sections were cutted with a diamond knife, mounted on (200 mesh) copper grids and contrastained with uranyl acetate and lead citrate (REYNOLDS, 1963). The grids were examined using Jeol TEM-100 C II at 80 KV.

Egg inoculation: 12 embryonated fowl eggs, 10-day-age were used for trials of viral isolation. Bacteria free suspension from liver and lungs was prepared by adding penicillin 10.000 lu/ml and streptomycin 10 mg/ml. Samples were left at room temperature for 2 hours in the antibiotic solution. Supernatant obtained after light centrifugation of the samples in antibiotic solution was inoculated into the alantoic cavity of 6-eggs. The eggs were held at 37°C until dead or dying after 5 days of the 2nd passage. Chilling of eggs at 4°C was conducted and the amnioallantoic fluid was harvested. Liver suspension from embryos was prepared and tested as well as aminoallantoic fluid for haemagglutination character, using type 0 human and chicken red blood cells as described by CHEN (1986).

Haemagglutination inhibition test (HI): Was performed after PU et al. (1985), by using convalescent serum and normal rabbit serum as a control.

Experimental animals: 10 healthy rabbits, 4-months-old obtained from Assiut private rabbit farm and 4 white-Swiss mice, 4-weeks-old obtained from the Laboratory Animal Unit, Faculty of Medicine, Assiut University were used for experimental infection.

Experimental design: For reproducing the disease four rabbits were inoculated intramuscularly with 1 ml bacteria free suspension of liver and lung. Another four rabbits were injected subcutaneously with the same suspension and dose. The rest two rabbits were used as control with complete separation from infected ones. Two mice were inoculated subcutaneously with 0.2 ml of bacteria free tissue suspension and the another two mice were left as uninoculated control. All animals were kept under observation for a maximum of 7 days post infection for the development of clinical signs and mortalities. Post mortem examination was performed on dead rabbits as well as sacrificed animals at the end of the experiment. Tissue specimens were taken for electron microscopy and haemagglutination test as previously mentioned.

# RESULTS

Bacterial isolation: Bacterial isolation from liver and heart blood of naturally dead rabbit revealed negative results.

Natural outbreak: About 200 rabbits of different breeds, from nine rabbit farms were examined. The age of the diseased animals ranged from 4 to 13 months. The disease was not observed in rabbits less than 2 months within the affected herds. The deaths were usually sudden, affecting several groups simultaneously. The mortality rates was 100% among a rabbit farm and it was 96.5% in another colony. The environmental conditions of the examined animals were markedly different including husbandry,

hygiene and nutrition. The disease invaded females, pregnant females and male rabbits. Infected rabbits died suddenly without any observed clinical manifestations. Occasionally, a haemorrhagic foamy discharge from the nostrils and vaginal haemorrhages were observed (Fig. 1a).

Post mortem examination of naturally dead rabbits revealed severe hypereamia of the blood vessels in the subcutaneous tissue, kidneys, lungs (Fig. 1b) and the cranial blood vessels (Fig. 1c). Moreover, petechial and echymotic haemorrhages were observed in the serosal surfaces of the stomach and intestine. In all cases, the liver was diffusely pale in colour with the congestion mainly concentrated at its borders (Fig. 1b). The trachea contained blood stained mucous.

Experimental infections: the incubation period of experimentally-infected animals ranged from 42 to 66 hours, with a mortality rate of 87.5% (7, out of 8 inoculated rabbit). Clinical symptoms as reluctance to move and anorexia with cyanosis of the visible mucosa and skin were observed. Recumbency and paddling movements of the limbs were the signs seen just before death, while depression of some rabbits was observed. It is worth to mention that, no foamy haemorrhagic discharge from nostrils and vagina was recorded. There were neither deaths in mice nor in control animals. The experimentally infected rabbits showed post mortem lesions similar to those described in naturally infected animals. However, no intestinal lesions were observed in those animals died at the early stages of infection.

Light microscopy: Light microscopical examination of semithin sections of the liver revealed various degenerative changes in the hepatocytes. These changes were in the form of cytoplasmic vacuolization (Fig. 2a) and appearance of fat globules in the cytoplasm of hepatic cells (Fig. 2b). These findings suggested fatty degeneration of the hepatocytes. Furthermore, apoptotic hepatocytes were frequently observed indicating high rate of cell death (Fig. 2b). The hepatic sinusoides were congested and the lining Kuppfer cells were swollen (Fig. 2b). The lungs showed congestion of the alveolar capillaries and desquamation of the alveolar lining cells (Fig. 2c). These cells were seen free in the alveolar lumen together with extravasated erythrocytes (Fig. 2d).

Electron microscopy: Revealed crystalline arrays of virus-like particles, were frequently found in the cytoplasm of hepatocytes. Virus was unenveloped and round in shape.

HA and HI tests: the using of virus antigen in tissue suspensions from both naturally and experimentally infected animals gave positive results with HA test, by use type 0 human and chicken erythrocyte. The agglutination titers (1/320 and 1/40 respectively) were higher on using extracts from the liver, lungs, spleen and kidneys than using extracts from the heart and brain. Using serum from convalescent animals gave positive haemagglutination inhibition test in comparison with serum from healthy rabbits.

Viral propagation: Multiplication of the virus in allantoic sac did not produce deaths in chicken embryos. Embryonic fluids and liver suspension from killed infected embryos proved negative HA test.

#### DISCUSSION

Viral haemorrhagic disease in rabbits as a highly pathogenic disease causing severe outbreaks in rabbitary was recently diagnosed in Assiut area. This was based upon the clinical picture of the disease which include sudden deaths with the occasionally presence, of haemorrhagic foamy discharge from the nostrils and vagina in adult female rabbits. Similar symptoms were previously described by LOLIGER et al. (1989); SOIKE et al. (1989) and XU and CHEN (1989) in rabbits. The obtained data revealed that the mortality rate of the disease was more than 85% among both naturally and experimentally infected rabbits. GRANZOW et al. (1989); OHLINGER et al. (1989) and SINGER et al. (1989) recorded 100% mortality rate of the disease in natural outbreaks. In the present study, the gross pathological findings were similar in both naturally and experimentally infected rabbits. These lesions were in the form of hyperemia of the kidneys, lungs and brain. Diffuse paleness of the liver was also a prominent feature. These observations were similarly described by KOLBL et al. (1990) and NOWOTNY et al. (1990).

Histopahology of the liver revealed vasculation and fatty degeneration of the hepatocytes and sinusoidal congestion. The lungs showed intense hyperemia of the alveolar wall, loss of the lining alveolar cells and alveolar hemorrhage. These data were consistent with other reports on RVHD (XU et al., 1985 and KOLBL et al., 1990). The pathological changes were believed to result from the viraemia with widespread circulatory dysfunction (XU et al., 1985). In this study, virions were demonstrated in the cytoplasm of hepatic cells by transmission electron microscopy suggesting that these changes were attributable to virus replication in the hepatocytes. The sudden death of animals may be due to multiple organ dysfunction associated with extensive pulmonary congestion and oedema, severe haemorrhagic pneumonia and generalized circulatory disorders of kidneys (XU and CHEN, 1989).

On the other hand, rabbit diseases causing sudden deaths like Pasteurellosis, staphylococcosis and mycotoxicosis were discarded by bacteriological work. Moreover, no deaths of inoculated mice (not susceptible to RVHD), were observed which exclude the presence of toxins in the suspension of liver and lungs of naturally dead animals. In similar studies concerning age susceptibility, our observations are in agreement with those described by GRANZOW et al. (1989); OHLINGER et al. (1989) and SINGER et al. (1989) who recorded that most outbreaks occurred among rabbit aged more than two months.

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The results of HA test on liver and lung suspensions using type 0 human and chicken erythrocytes gave positive results with high titer (1/320 and 1/40) respectively.

This is in a ccordance with findings of FRESCURA et al. (1989). Conversely, SOIKE et al. (1989); XU et al. (1988) and XU & CHEN (1989), obtained a lower HA titers using chicken and sheep erythrocytes. The results of electron microscopy revealed the presence of icosahedral and round shape viral particles mainly distributed in the cytoplasm. The morphology of the viral particles was similar to calicivirus aetiology. These results coincide with the findings of other authors (FRESCURA et al., 1989; CAPUCCI et al., 1990; OHLINGER et al., 1990). It is worth to record here that no satisfactory methods have yet been found for culturing RVHD virus. Although replication of RVHD virus in chicken embryo failed. These findings in our study resemble those described by (XU et al., 1988 and NOWOTNY et al., 1990).

Finally it can be concluded that RVHD is one of the most severe viral diseases affecting rabbitary. Sudden deaths and high mortality rates of the disease are major factors of the resulting economic losses. Moreover, trials for diagnosis and follow-up of this disease all-over the country together with the preparation of inactivated vaccine as a control measure must be also considered.

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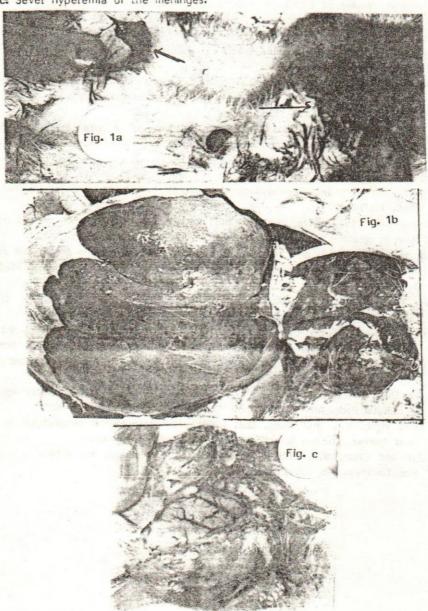
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# LEGENDS

Fig. 1a: Naturally infected rabbit showing hemorrhagic discharge from the vaginal Fig. 1b: Diffuse paleness of the liver and congestion of the lungs.

Fig. 1c: Sever hyperemia of the meninges.



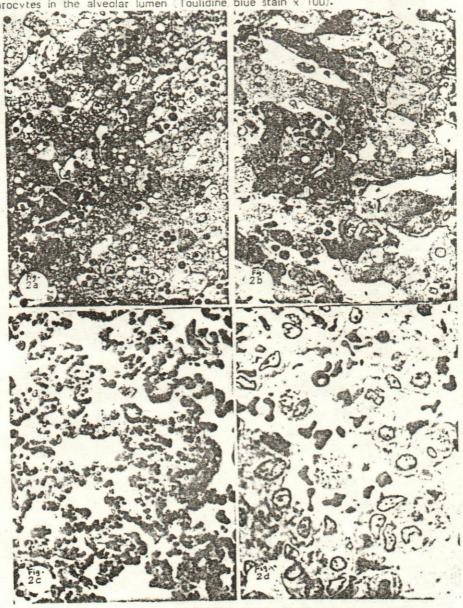
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Fig. 2a: Experimentally infected rabbit liver showing cytoplasmic vacuolization of hepatocytes, sinusoidal congestion and swelling of the lining Kuppfer cells (Toulidine blue stain x 25).

Fig. 2b: Fatty degeneration of hepatocytes (Toulidine blue stain x 25).

Fig. 2c: Lung of experimentally infected rabbit showing congestion of the alveolar wall and loss of the alveolar lining cells (Toulidine blue stain x 25).

Fig. 2d: Higher magnification to demonstrate alveolar cells and extravasated erythrocytes in the alveolar lumen (Toulidine blue stain x 100).



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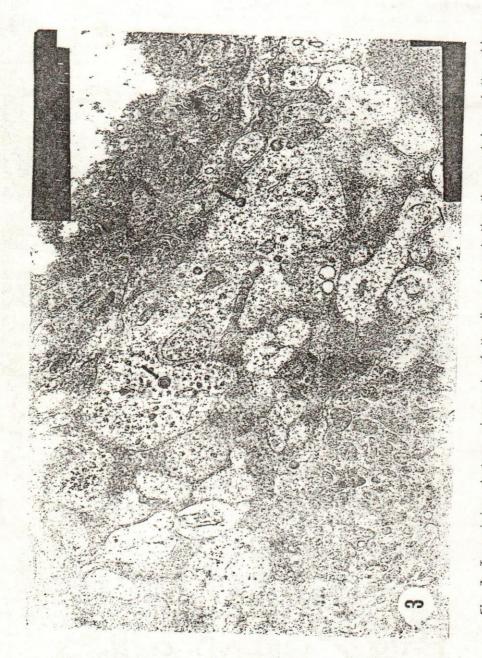


Fig. 3: Transmission electron micrograph of the liver demonstrating the morphology of the virions in the cytoplasm of hepatic cells (uranyl acetate and lead citrate, x 40,000)